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Proliferation and AKT activity biomarker analyses after Capivasertib (AZD5363) treatment of patients with ER+ invasive breast cancer (STAKT)

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Running title: Capivasertib rapidly targets key AKT pathway biomarkers

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Conflicts of interest:

JFRR has received consulting fees from, and performed contracted research on behalf of, AstraZeneca, Bayer, Novartis, and Oncimmune, has given expert testimony for AstraZeneca, and holds stock with Oncimmune; REC has received consulting and/or speaker fees from Amgen, Astellas, Eisai, Genomic Health, Inbiomotion, and Scancell and is a patent holder for a biomarker developed by Inbiomotion; K-LC has received research funding from AstraZeneca and served as an advisory board member for Genomic Health; CH has received consultancy fees from Pfizer; RL and PF have

received funding from the University of Nottingham; JMWG has received research funding from AstraZeneca; SYAC, MC, ECB, LK,JPOL, MP, PR, GS, and AF are employees of and own stock with AstraZeneca; AE, AS, DR, SA, AJ, KH, PR, RD, and PF have no conflicts to declare.

Statement of translational relevance

Capivasertib (AZD5363) monotherapy previously demonstrated anticancer activity in Phase I trials in patients with advanced solid tumors, particularly those whose tumors harbored an AKT mutation. In the short time frame that window-of-opportunity studies permit, the STAKT study reported here determined that in primary breast cancers, capivasertib at the recommended monotherapy dose (480 mg twice daily [bid]) rapidly modulated the AKT pathway (after 4.5 days of treatment), as evidenced by significant decreases from baseline versus placebo in biomarkers of the AKT pathway (including pGSK3β and pPRAS40), and reduced cell proliferation (Ki67). Biomarker modulation was also observed at lower capivasertib doses of 240 and 360 mg bid, and the inhibitory effects were dose and concentration dependent. Overall, the STAKT study provides further evidence that capivasertib has the potential to be an effective oral anticancer therapy via its impact on proliferative AKT signaling in patients with estrogen-receptor-positive breast cancer.

Abstract

Purpose: The STAKT study examined short-term exposure (4.5 days) to oral selective pan-AKT inhibitor capivasertib (AZD5363) to determine if this drug can reach its therapeutic target in sufficient concentration to significantly modulate key biomarkers of the AKT pathway and tumor proliferation.

Methods: STAKT was a two-stage, double-blind, randomized, placebo-controlled, 'window-of-opportunity' study in patients with newly diagnosed ER+ invasive breast cancer. Stage 1 assessed capivasertib 480 mg bid (recommended monotherapy dose) and placebo, and stage 2 assessed capivasertib 360 and 240 mg bid. Primary endpoints were changes from baseline in AKT pathway markers pPRAS40, pGSK3β and proliferation protein Ki67. Pharmacologic and pharmacodynamic properties were analyzed from blood sampling, and tolerability by adverse-event monitoring.

Results: After 4.5 days' exposure, capivasertib 480 mg bid (n=17) produced significant decreases from baseline versus placebo (n=11) in pGSK3β (H-score absolute change –55.3, *P*=0.006) and pPRAS40 (–83.8, *P*<0.0001), and a decrease in Ki67 (absolute change in percentage positive nuclei: –9.6%, *P*=0.031). Significant changes also occurred in secondary signaling biomarker pS6 (–42.3, *P*=0.004), while pAKT (and nuclear FOXO3a) also increased in accordance with capivasertib's mechanism (pAKT: 81.3, *P*=0.005). At doses of 360 mg bid (n=5) and 240 mg bid (n=6), changes in primary and secondary biomarkers were also observed, albeit of smaller magnitude. Biomarker modulation was dose and concentration dependent, and no new safety signals were evident.

Conclusions: Capivasertib 480 mg bid rapidly modulates key biomarkers of the AKT pathway and decreases proliferation marker Ki67, suggesting future potential as an effective therapy in AKT-dependent breast cancers.

Introduction

Components of the AKT pathway (also known as the PI3K/AKT/mTOR signaling pathway) play a fundamental role in tumor cell survival, proliferation and death (Supplementary Figure 1) (1). Mutations in signaling components can cause aberrant activation of the pathway, leading to the development of numerous solid and hematologic malignancies (1-3) and resistance to endocrine therapies (4, 5). Mutations in *PIK3CA*, *AKT1* and *PTEN* are prevalent in estrogen-receptor-positive (ER+) breast cancer (6), indicating that this pathway is important in ER+ breast cancer, which is further augmented by the observation of a reciprocal feedback between the ER and PI3K/AKT signaling pathways.

Capivasertib (AZD5363) is a new oral selective AKT1–3 inhibitor that demonstrated promising clinical activity and a tolerable safety profile as monotherapy treatment in a Phase I study in heavily pre-treated (median of five prior regimens) patients with *AKT1* E17K mutant metastatic solid cancers, with the strongest signal of activity observed in ER+ breast cancers (7). In cancer model systems, the inhibitor blocks the AKT pathway, depleting phosphorylation of pathway proteins GSK3β, PRAS40, and S6 and tumor cell growth (8). In preclinical experiments, reductions of 50–80% in phosphorylated PRAS40 (pPRAS40) and 40–70% in pGSK3β during the capivasertib dosing period were sufficient to cause significant antitumor activity in several xenograft models (eg 100 mg/kg twice daily [bid] in the BT474c xenograft model) (8). For Ki67, information was drawn from two previous pre-surgical studies of endocrine therapy in ER+ breast cancer (9, 10). The change in Ki67 in one of these, an endocrine study (NCT00259090), was a >80% decrease in all treated groups with clear statistical significance (10).

Successful drug development is critically dependent on an understanding of drug pharmacodynamics, including knowledge of the drug's rapidity of action and effect on molecular treatment targets. In this regard, the STAKT trial (NCT02077569) is the first two-stage, pre-surgical, window-of-opportunity biomarker study to focus on assessing the effects of a range of capivasertib monotherapy doses on key markers of the AKT pathway and expression of a protein strongly associated with tumor cell proliferation and growth (Ki67) in patients with newly diagnosed ER+ breast cancer. These markers were used to characterize the degree of biological activity and, thus, treatment potential in primary breast cancer arising from the inhibition of AKT signaling across a range of capivasertib doses.

The STAKT study aimed to help define the optimal dose of capivasertib for future studies, as well as aid further understanding of its impact on the AKT pathway and biomarker identification. Stage 1 of the study assessed the pharmacodynamic (PD) and pharmacokinetic (PK) effect and tolerability of capivasertib 480 mg bid versus placebo. Stage 2 examined the same, but at lower doses (320 and 240 mg bid) of capivasertib. It was hoped that exploring a range of doses in the study would also enable predictive model-based techniques to be developed to help identify an efficacious dosing range and dose reduction strategies for potential use in future stages of clinical development in breast cancer.

Here, data are presented from STAKT stages 1 and 2 that reveal the targeted effect of capivasertib on selected tumor and cell proliferation biomarkers, the PD properties of the dose– and exposure–response relationship, and tolerability from short-term exposure in patients with breast cancer.

Patients and methods

Key eligibility criteria

Eligible patients were females aged ≥18 years with histologic confirmation of ER+ invasive breast carcinoma (stage 1–3 or stage 4 breast cancer with primary tumor in the breast amenable to biopsies) and tumors large enough to provide tissue for the biomarker assays. Additional key eligibility criteria included: subsequent standard of care determined to include chemotherapy, with or without surgery (as such treatment was considered to carry a greater risk of genotoxicity compared with exposure to capivasertib); WHO performance status 0–1 with no deterioration over the previous 2 weeks; and hepatic, renal, cardiac, lung and gastrointestinal functions within normal limits, except if liver metastases were present, in which case liver enzyme levels could be up to three times the upper limit of normal. To reduce the potential risk of the drug exacerbating abnormal glucose profiles (11), patients with type 1 or 2 diabetes mellitus (irrespective of management), fasting glucose ≥7 mmol/L or glycated hemoglobin (HbA_{1c}) ≥64 mmol/mol or ≥8% at screening were excluded from participating in this study. Patients had no known history of hypersensitivity to the active ingredient or inactive excipients of capivasertib.

Study conduct

All patients provided written informed consent. The National Research Ethics Service Committee East Midlands – Northampton Research Ethics Commitee (REC) approved the protocol. The STAKT study (trial registration ID: NCT02077569; REC ID: 13/EM/0112; Clinical Trial Authorization reference: 03057/0057/001) had local National Health Service (NHS) R&D approval and was conducted in accordance with the Declaration of Helsinki, Good Clinical Practice and the Department of Health Research Governance Framework for Health and Social Care 2005. The study was also performed

in accordance with the Cancer Research UK Guidelines for Scientific Conduct (12). An independent data monitoring committee (IDMC) reviewed emerging data and, in particular, the results of stage 1 to approve the continuation of the study into stage 2. Trial oversight was provided by an independent trial steering committee on behalf of the funders and sponsors. The trial management group was responsible for the running of the trial. Tayside Clinical Trials Unit was responsible for the day-to-day management of the trial and undertook the data analyses in accordance with the statistical analysis plan for the study.

Study design and treatment

STAKT was a multicenter, two-stage, double-blind, randomized, placebo-controlled, paired-biopsy, biomarker study conducted in NHS hospitals throughout the UK. The two-stage study format was designed to obtain early evidence that the drug is pharmacokinetically and pharmacodynamically active at the 480 mg bid dose, which, based on initial tolerability, PK and preliminary efficacy data from a previous Phase I study (NCT01226316) (13), was the most likely dose and schedule to be used in clinical studies.

In stage 1, eligible patients were randomized (1:1) to either capivasertib 480 mg bid or placebo for 4.5 days. In stage 2, patients were randomized (1:1) to capivasertib 360 or 240 mg bid for 4.5 days (Supplementary Figure 2). Enrollment in stage 2 was planned to commence seamlessly upon completion of stage 1, dependent on, in the opinion of the IDMC, the 480 mg bid dose producing a depletion to a pre-specified margin of ≥50% over placebo for the primary biomarkers pPRAS40, pGSK3β (both key indicators of AKT signaling) and Ki67. In fact, there was a short break in patient recruitment between stages 1 and 2, supported by the study IDMC, as the study team made the decision to

remove an originally planned placebo arm for stage 2 (protocol amendment approved by the REC and the Medicines and Healthcare Products Regulatory Agency in December 2015) because of unforeseen recruitment challenges. Changes in the lower doses of capivasertib were then assessed against baseline rather than placebo.

Patients received capivasertib for 4.5 days prior to their scheduled surgery or 6.5 days prior to scheduled chemotherapy (the final morning dose was taken on the day of surgery, which accounts for the final 0.5-day period). Ultrasound-guided tumor core biopsies were taken prior to the first dose and after 4.5 days of dosing (within 12 hours after the last dose). Additional 'triple blinding' was performed by blinding laboratory staff to treatment arm and time of biopsy (baseline or after 4.5 days). Biopsy samples were formalin fixed and paraffin embedded and immunohistochemically stained using the pre-validated assays detailed in Supplementary Table 1, and tumor epithelial staining was assessed by H-scoring the primary biomarkers pGSK3B and pPRAS40 by consensus of two expert assessors. H-score was calculated as the sum, (% weak [1+]) + (% moderate [2+] x 2) + (% strong [3+] x 3), of staining localized in the cytoplasm and/or nucleus. Ki67 was evaluated by percentage nuclear positivity only. Biopsy pairs were sectioned together and analyzed in the same immunostaining assay, with a positive internal control included in every assay for quality control purposes. Blood samples for PK were scheduled at: pre-dose; 2, 4, and optional 6 and 8 hours after the first dose on day 1; and after the last dose on day 5, as close to the time of the on-treatment biopsy as possible.

Study objectives

The primary objective of STAKT was to compare the AKT pathway biomarker and antiproliferative effect of 4.5 days' treatment with three dose levels of capivasertib.

Primary endpoints were changes from baseline for the active phosphorylated forms of the selected primary biomarkers, pPRAS40 and pGSK3β, and Ki67. Secondary endpoints included changes in additional biological markers associated with the AKT pathway, comprising tumor epithelial staining for pAKT (cytoplasmic/nuclear/membrane), phosphorylated ribosomal protein S6 (cytoplasmic) and FOXO3a expression (nuclear and/or cytoplasmic), as well as an assessment of tolerability through monitoring of adverse events (AEs) with Common Terminology Criteria for Adverse Events (CTCAE) version 4 (graded as mild, moderate or severe). Additionally, relationships between primary biomarkers and capivasertib exposure (dose and plasma concentration) were explored *post hoc* to help identify a dosing range for potential use in later clinical development.

Statistical design

Up to 60 patients were planned to be recruited in each of stages 1 and 2. Twelve patients per arm with evaluable biomarker data would give 98% power to detect a difference of 30% (change from baseline) for pPRAS40 and pGSK3β levels between the treatment arms, at the 5% significance level. Assumptions for Ki67 in this study were for 80% power and a two-sided significance level of 5%. With 12 evaluable patients, the study would be powered to show a 50% reduction in Ki67.

Statistical analysis methods

The biomarker population consisted of all patients with evaluable biomarker data at baseline and evaluable pre- and on-treatment biopsies. Evaluable patients were defined as having both pre- and on-treatment biopsy samples with a minimum of 100 tumor epithelial cells for H-score assessment for both pGSK3β and pPRAS40, as well as a minimum of 500 tumor epithelial cells to count for percentage positivity for Ki67. In

addition, the tumor had to express all three primary endpoint biomarkers in the pre-treatment biopsy (defined as measureable percentage positivity for Ki67 nuclear staining [any score greater than zero is acceptable] and a total H-score, ie cytoplasm + nuclear, of ≥10 for each of pPRAS40 and pGSK3b), and patients also should have received the full planned 4.5-day dose of capivasertib treatment.

An analysis-of-covariance model was fitted to the biomarker data, including terms for treatment and adjusting for baseline biomarker level. For each biomarker, changes in expression between the matched baseline and on-treatment biopsies were evaluated and compared with changes seen in the placebo group (comparison with placebo in stage 1 only). Both absolute and percentage changes from baseline were recorded. Stage 2 comparisons were against baseline biomarker level. The strength and direction of association between biomarkers were assessed by Spearman's rank correlation coefficient.

Dose– and exposure–response relationships were compared for three exposure variables: dose level given (ie 240 mg bid, as opposed to the total daily dose of 480 mg per day); observed maximum capivasertib plasma concentration (C_{max}) on day 1; and capivasertib plasma concentration on day 5. Linear and E_{max} models were compared for each exposure variable, and the Akaike information criterion was used as a goodness-of-fit diagnostic (14).

The safety population consisted of all patients receiving ≥1 dose of study drug and was assessed by the incidence and severity of treatment-emergent adverse events (TEAEs) reported within 7 days following the first dosing of capivasertib, or up to the time of start of chemotherapy if earlier.

Results

Patient disposition

In total, 36 patients from 12 participating hospitals were randomized in stage 1 (19 patients to capivasertib 480 mg bid, 17 patients to placebo), and 12 patients in stage 2 (7 patients to capivasertib 240 mg bid, 5 patients to capivasertib 360 mg bid). All 48 randomized patients (36 patients from stage 1, 12 patients from stage 2) received at least one dose of study treatment and were evaluable for safety analysis. Eight patients were deemed not evaluable for biomarker analysis in stage 1. All patients were deemed evaluable in stage 2, giving a total evaluable biomarker population of 40 patients.

Of the seven patients randomized to capivasertib 240 mg bid in stage 2, one was subsequently found not to meet the pre-specified eligibility criterion of a total H-score of ≥10 for pGSK3β (total score was 6) and pPRAS40 (total score was 5) staining in the pre-treatment sample. This was identified after the stage 2 data lock; hence, this patient was excluded from the biomarker analysis. Therefore, a final total of 39 patients were evaluable in the biomarker population (28 patients from stage 1, 11 patients from stage 2) according to the pre-defined evaluability criteria. No notable differences between the baseline characteristics in the patient populations enrolled across stages 1 and 2 were discerned (Table 1).

Changes in biomarkers

Significant reductions in the absolute change measurements in H-score for the primary biomarkers were observed for the 480 mg bid dose versus placebo: –55.3 (P=0.006) for total pGSK3β and –83.8 (P<0.0001) for total pPRAS40 (Table 2). The absolute percentage reduction in nuclei staining positive for Ki67 was also significant, at –9.6 (P=0.031). The percentage change from baseline at the 480 mg bid dose versus

placebo was also significant for total pGSK3β (–39% [*P*=0.006]) and total pPRAS40 (–50% [*P*<0.0001]). Reductions from baseline (absolute and percentage) were also observed for pGSK3β and pPRAS40 at the 360 mg bid and 240 mg bid doses. The secondary biomarkers pAKT and pS6 also showed significant change from baseline in H-score for the 480 mg bid dose versus placebo, but FOXO3a did not (Table 2).

However, when the contribution to total H-score from cytoplasmic staining was separated from nuclear staining for the biomarkers whose total H-scores were derived from nuclear plus cytoplasmic staining (pGSK3β, pPRAS40, pAKT and FOXO3a), it was noted that FOXO3a showed a significant decrease in cytoplasmic H-score (absolute change –46.2, *P*<0.0001 vs placebo), while there was a corresponding substantial increase in nuclear H-score for this marker (absolute change 79.3, *P*=0.0009 vs placebo) at the 480 mg dose level. Such a difference in staining profile for different cellular compartments was not observed for the other biomarkers (Table 2).

An immunohistochemistry assay for cleaved caspase-3, as a measure of apoptosis, was additionally performed, and the percentage positivity was gauged in a total population of 500 and, where possible, 3000 tumor cells. Absolute and percentage changes were assessed by analysis of variance (ANOVA) adjusted for treatment and baseline values. Only one of eight ANOVA analyses (considering percentage apoptosis in 3000 cells adjusted for baseline) gave a *P* value of <0.05 (data not shown).

A ‘heat map’ showing the changes in the primary and secondary biomarkers for each patient in the three capivasertib groups, as well as the placebo group, is shown in Figure 1a. Compared with placebo, more substantial and frequent decreases were seen with the 480 mg dose for Ki67 and the AKT pathway markers pGSK3β, pPRAS40 and pS6,

with 13/17 patients showing some change in all three primary biomarkers. Increases in turn were more common with such treatment for pAKT (12/17 patients) and nuclear FOXO3a expression (13/17 patients). At doses of 360 and 240 mg bid, declines in the biomarkers pGSK3β, pPRAS40 and pS6 were also observed, albeit of smaller magnitude for the primary markers than seen with the 480 mg dose (particularly for Ki67; Table 2). Increases were again noted for pAKT and nuclear FOXO3a. Representative immunohistochemistry staining images for both primary and secondary biomarkers at baseline and on treatment can also be seen in Figure 1b, showing declines in Ki67, pGSK3B, pPRAS40 and pS6, and increases in pAKT and nuclear FOXO3a, with the 480 mg dose that are not apparent with placebo.

Correlation coefficient analyses

A correlation coefficient analysis (Spearman's rank) of the percentage changes in biomarkers at the 480 mg bid dose (Figure 2) indicated significant positive correlation between changes in pGSK3β (total) and Ki67 (nuclear) (R=0.52, *P*=0.031), and between pGSK3β (total) and pS6 (cytoplasmic) (R=0.54, *P*=0.025). Negative correlations were determined between FOXO3a (nuclear) and Ki67 (nuclear) (R=0.75, *P*<0.0001), FOXO3a (nuclear) and pGSK3β (R=0.71, *P*=0.0014), and FOXO3a (nuclear) and pS6 (R=0.61, *P*=0.0092). These correlations observed at the 480 mg bid dose were not replicated in the placebo group (Figure 2). Correlation coefficients for the lower doses were not robust, most likely because of the small patient numbers in these groups, although a reduced PD effect cannot be ruled out.

Pharmacokinetics

The capivasertib C_{max} was generally observed 2 hours post-dose. There was a trend towards increasing C_{max} and concentration on day 5 with increasing dose (Figure 3). The

variation in concentration on day 5 is influenced by the variability in sampling times, which is noted in the discussion.

Dose– and exposure–response relationships

Biomarker modulation was dose and concentration dependent. The dose–response relationship for percentage change from baseline could be described by a non-linear (E_{max}) model for all primary biomarkers (Figure 4). Similar correlations were observed for the change in the biomarkers and PK exposure (C_{max} on day 1 or concentration at the time of biopsy on day 5).

Tolerability

In total, 78 TEAEs (ie within 7 days of first dosing or before scheduled chemotherapy) were reported in 27 of 31 (87%) patients receiving capivasertib (all doses), and 23 TEAEs in 10 of 17 (59%) patients receiving placebo (Table 3). Among the 27 patients receiving capivasertib (all doses) and reported to have a TEAE (all grades – mild, moderate or severe), diarrhea was observed most frequently, with 13 (17%) events out of a total of 78, followed by nausea with eight events out of 78 (10%).

One patient (1.7%) receiving capivasertib 480 mg was reported to have a decreased ejection fraction. No hyperglycemic TEAEs were reported in the capivasertib-treated patients. Three patients had an AE assessed by the investigator as ‘possibly’ causally related to the study drug (one in each patient): 480 mg bid, atypical migraine; 360 mg bid, neutropenia; and 240 mg bid, neutropenia. All three patients subsequently recovered from the AE.

Three treatment-emergent serious adverse events (SAEs) were recorded at the 480 mg bid dose (delayed recovery from anesthesia, nausea, vomiting) and two at the 340 mg bid dose (dizziness, nausea). No SAEs were recorded in the 240 mg bid or placebo groups. No patients discontinued capivasertib (any dose) because of an AE.

Discussion

Window-of-opportunity studies performed in the time between diagnosis and standard-of-care treatment, in which patients are briefly exposed to an investigative compound, can facilitate early understanding of the PD and dose/exposure–response effects of a drug and may confirm identification of predictive response markers for retrospective analyses of ongoing or completed clinical trials (15). Equally, such studies provide a unique opportunity to monitor drug mechanism *in vivo*, including providing evidence of hit on the target pathway and its important cellular endpoints.

The principal research questions addressed in the STAKT study reported here were whether the new selective pan-AKT inhibitor capivasertib, particularly at the recommended monotherapy dose of 480 mg bid, could reach its therapeutic target AKT pathway in primary breast cancer patients, and if so, whether this was to the extent necessary to produce anticancer efficacy; these were assessed by the effects on markers indicative of functional AKT signaling and tumor proliferation, respectively. Our results show that capivasertib 480 mg bid, after 4.5 days of treatment, does indeed reach its therapeutic AKT pathway target in such patients, as evidenced by statistically significant decreases from baseline compared with placebo in activity (phosphorylation) of the pathway biomarkers GSK3β and PRAS40, as well as pS6, accompanied by significant decreases in tumor Ki67 (Table 2). Pathway biomarker modulation was also observed at the lower doses of 240 and 360 mg bid, albeit generally to a lesser degree, indicating that the inhibitory effects of capivasertib were dose and concentration dependent.

A heat map of the degree of biomarker modulation (Figure 1) further revealed that most patients receiving capivasertib 480 mg bid showed a reduction from baseline in the

primary biomarkers pGSK3β and pPRAS40, as well as in Ki67, and a (high) degree of correlation in the modulation of biomarkers within the same patient. By comparison, in the placebo group, changes were smaller in magnitude and more randomly distributed between increases and decreases, indicating no direct, substantive effects in the placebo group, contrasting with capivasertib, which was having a targeted modulating effect across multiple biomarkers in the AKT pathway. At the 480 mg bid dose, the heat map showed that marked primary biomarker changes were often seen in the same individuals, as opposed to the placebo group, in which such changes tended to be spread across several patients. Correlations between changes in pGSK3β and pS6, and between pGSK3β and Ki67, were identified for the 480 mg bid dose that were further suggestive of pathway element interplay, its impact on proliferation, and its targeting by capivasertib. Also, in all the capivasertib-treated groups, changes in multiple primary and/or secondary endpoint signaling pathway markers were seen in the majority of individuals. In total, these observations are indicative of capivasertib reaching its intended target as a controlled 'hit' down the whole AKT pathway in breast cancers. At doses of 360 and 240 mg bid, similar observations can be made to those at 480 mg, albeit of smaller magnitude for the primary markers (particularly for Ki67, whereby decreases were also less frequent), again suggesting that after 4.5 days' dosing, capivasertib was reaching its intended signaling pathway target in a consistent manner.

The secondary biomarkers analyzed (pAKT, pS6 and FOXO3a) also showed significant changes in this study that are consistent with inhibition of the AKT pathway. The observed induction of pAKT is consistent with previous preclinical and clinical data; capivasertib increases phosphorylation of AKT itself, and it has been reported that this is due to the protein being held in a hyperphosphorylated but catalytically inactive form as a consequence of compound binding (8, 13, 16). Results were obtained from paired

biopsies collected during two previous Phase I studies (NCT01226316 and NCT01353781), albeit in metastatic solid malignancies (including those with *PIK3CA* mutation). These Phase I studies also showed downregulation of PD biomarkers (eg pPRAS40 and pGSK3b) following capivasertib treatment, increased phosphorylation levels of AKT (consistent with ATP competitive mechanism of action), and inhibition of FOXO nuclear translocation (13, 17).

In contrast to these Phase I studies, the STAKT study was more controlled, ie paired samples were collected at similar time points and from patients with similar disease and a placebo control group was included. The STAKT study results we report are consistent with the data from the Phase I studies and demonstrate the PD effects more robustly and for the first time in primary breast cancers. For FOXO3a, the large decrease in cytoplasmic staining and corresponding large increase in nuclear staining after 4.5 days' exposure seems likely to be a result of redistribution of FOXO3a within the cancer cell as a consequence of upstream changes in AKT signaling pathway activity. This is consistent with the data in paired biopsies from solid tumors in the capivasertib Phase I study (13) and validates the preclinical observation of induction of nuclear accumulation of FOXO3a in BT474c breast cancer cells (HER2 amplified *PIK3CA* mutant) exposed to capivasertib (18). It has been reported that AKT-pathway-driven phosphorylation of FOXO3a is arrested by AKT inhibition (18), permitting translocation of FOXO3a to the nucleus; in keeping with this, inverse associations were seen in our study between changes in nuclear FOXO3a and the pathway signaling markers pGSK3β and pS6. Once inside the nucleus, as a transcription factor, FOXO3a is able to initiate expression of the tumor suppressive genes *BIM*, *FasL* and *p27*, which collectively can induce cell cycle arrest and so may contribute to proliferation inhibition with the drug and/or apoptosis (18). The effect of capivasertib on such tumor proliferation, within the

treatment window of 4.5 days, is clearly seen in the Ki67 results here, and changes in nuclear FOXO3A with 480 mg dose also showed an indirect correlation with changes in Ki67. The impact of the drug on apoptosis, whereby cell survival can be a further consequence of AKT signal transduction, has not been clearly shown within the short time frame of the STAKT study by the cleaved caspase-3 staining results. Future translational studies should look to address this question.

Peak plasma concentration for capivasertib was generally observed 2 hours post-dose, and there was a trend towards increasing C_{max} and concentration on day 5 with increasing dose. Variability in time of sampling after dose caused variation in day 5 concentrations between patients that contributed to the large overlap noted between dose groups (Figure 3). The PK data reported here are consistent with the emerging PK profile for capivasertib that indicates a median time to C_{max} of 2 hours (range 0.5–6 hours), with a terminal half-life of approximately 10 hours (range 7–15 hours) after the first dose (11, 13, 17). Weak dose–response relationships (percentage change from baseline) were identified for the two primary biomarkers (pGSK3β and pPRAS40) and Ki67. Generally, the correlation between the primary biomarkers and PK exposure was similar to the correlation with dose.

The safety profile of capivasertib was previously reported, and the most frequently reported side effects are gastrointestinal events (diarrhea, vomiting, nausea), fatigue, hyperglycemia and maculopapular rash (7, 11, 13). The safety assessment of capivasertib in the STAKT study reported was, by nature of a window-of-opportunity study, short in comparison with prior and ongoing studies of the compound. The TEAEs and SAEs observed in STAKT were as previously reported for capivasertib, and no new safety signals became evident.

Since the STAKT trial was completed, the Phase II study (PAKT – NCT02423603) has reported improved progression-free survival (PFS) and overall survival (OS) from the combination of capivasertib and paclitaxel as first-line therapy in patients with triple-negative breast cancer; an enhanced effect on PFS was observed in patients with mutations in the *PIK3CA*, *AKT1* or *PTEN* genes (19). More recently, in a Phase II, randomized, double-blind, placebo-controlled study (FAKTION (20) – NCT01992952), capivasertib in combination with fulvestrant significantly improved PFS in patients with advanced ER+ human epidermal growth factor receptor 2 negative (HER2–) breast cancer previously treated with aromatase inhibitors, with an observed OS improvement of approximately 6 months, although this was not statistically significant (37% OS data maturity). The successful development of a targeted AKT inhibitor such as capivasertib, either as monotherapy or in combination, may provide a new treatment option in breast cancer that helps circumvent endocrine therapy resistance from aberrant activation of the pathway (21). In a separate Phase II trial (BEECH – NCT01625286), adding capivasertib to weekly paclitaxel did not prolong PFS in a population of patients with advanced ER+/HER2− breast cancer or in a subpopulation whose tumors harbored a *PIK3CA* mutation (22). Notably, no concomitant endocrine therapy was permitted during the BEECH study. The STAKT translational study, with the PK/PD data showing that capivasertib is able to reach its therapeutic target in sufficient concentration to significantly modulate key biomarkers of the AKT pathway and tumor proliferation, provides biological support for the improved clinical outcomes seen in a likewise hormone-receptor-positive tumor population in the FAKTION trial.

Study limitations

The small sample size in stage 2, from unforeseen recruitment challenges, was mitigated by scientific rationale endorsed by ethical approval to withdraw the placebo arm in stage 2: it was agreed that assessment of dose-related effects could be achieved by comparing on-treatment PD results with baseline in the three dose groups, and that the placebo arm in stage 2 could be dropped. Biomarker changes after 4.5 days were therefore assessed against baseline biomarker levels in stage 2. This was different from stage 1, in which changes in biomarkers were compared with changes in the placebo arm. The reduced patient numbers in stage 2 also meant that robust statistical analyses of biomarker expression between the three doses was not possible. A second limitation was that the mutational status of these tumors was not assessed; therefore, it is not clear at this time whether the extent of the PD effect of capivasertib monotherapy is associated with alterations in key genes of the pathway (ie *PIK3CA*/*AKT1*/*PTEN*). This warrants further investigation.

Conclusions

The STAKT study has shown that capivasertib causes dose- and concentration-dependent effects on primary endpoint markers of its target AKT pathway (pGSK3β and pPRAS40) and the proliferation marker, Ki67, after only 4.5 days' exposure. To our knowledge, this is the first such study with this drug in primary breast cancer and is also the shortest pre-surgical window-of-opportunity study in breast cancer to show a significant decrease in tumor proliferation with a targeted therapy, which also exemplifies the effectiveness of such studies for investigating novel potential treatment compounds. Changes in secondary markers in the pathway (eg pAKT, FOXO3a and pS6) were also in accordance with the drug targeting this pathway. Correlations between a number of

tumor biomarkers were identified for capivasertib 480 mg bid (13). These correlations were in keeping with the drug's expected mechanism of inhibitory impact on the AKT signaling pathway, as were changes in the primary and secondary endpoints. Biomarker modulation was also observed at the lower capivasertib doses of 240 and 360 mg bid, although statistical analysis was limited by the small sample size at the lower doses.

The data presented confirm that capivasertib at the recommended monotherapy dose (480 mg bid) rapidly modulates the AKT pathway, and the significant resultant decrease in tumor Ki67 also raises the potential that it may be an effective anticancer therapy in AKT-dependent breast cancers. These findings, together with the positive results in the Phase II randomized trial FAKTION, support further development of capivasertib in patients with hormone-receptor-positive metastatic breast cancer.

Acknowledgments

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Tables

Table 1. Characteristics of patients at study entry (biomarker analysis set)

	Stage 1		Stage 2	
Clinical characteristics	**Placebo**	**Capivasertib 480 mg bid**	**Capivasertib 360 mg bid**	**Capivasertib 240 mg bid**
	(n=11)	**(n=17)[a]**	**(n=5)**	**(n=6)[b]**
Mean age, years (SD)	48.5 (7.2)	51.3 (9.6)	52.6 (12.9)	58.5 (11.1)
Mean BMI, kg/m^2 (SD)	27.3 (6.6)	28.8 (4.9)	31.5 (4.1)	27.4 (4.5)
Mean weight, kg (SD)	75.6 (17.9)	78 (12.9)	86.2 (11.4)	72.7 (9.4)
Mean height, m (SD)	1.7 (0.07)	1.7 (0.05)	1.7 (0.03)	1.6 (0.1)
Ethnicity, n (%)				
White (Caucasian)	10 (90.9)	17 (100)	5 (100)	6 (100)
Black (including Afro-Caribbean)	1 (9.1)	0	0	0

[a]Nineteen patients were randomized to capivasertib in stage 1, of whom 17 were evaluable for biological endpoints (two patients did not take all study medication and were therefore considered unevaluable for biological endpoints but were included in the safety analyses); [b]Seven patients were randomized to capivasertib in stage 2 (240 mg bid group) and included in the primary analysis. One patient was subsequently found not to meet the pre-specified eligibility criterion of a total H-score of ≥10 for pPRAS40 (total score was 5) and pGSK3β (total score was 6). BMI, body mass index; SD, standard deviation

Table 2. Analysis of change from baseline for the primary and secondary biomarkers

A) 480 mg bid versus placebo (stage 1)

			Capivasertib 480 mg bid (n=17)	Mean difference in change between groups (mixed model)[a]	
			Type of change vs baseline	Adjusted (95% CI)[b]	*P* value vs placebo arm
Primary					
Ki67 (% cells positive)					
	Nuclear	Absolute		–9.6 (–18.3, –0.98)	0.031
		%		–23.4 (–59.1, 0.29)	0.052
pGSK3β (H-score)					
	Total	Absolute		–55.3 (–93.4, –17.7)	0.006
		%		–39.0 (–65.5, –12.5)	0.006
	Cytoplasmic	Absolute		–53.6 (–90.5, –16.8)	0.006
		%		–39.2 (–65.8, –12.7)	0.006
	Nuclear	Absolute		–2.8 (–5.9, 0.19)	0.065
		%		–36.5 (–74.4, 1.35)	0.058
pPRAS40 (H-score)					
	Total	Absolute		–83.8 (–111.6, –56.0)	<0.0001
		%		–50.2 (–68.7, –31.7)	<0.0001
	Cytoplasmic	Absolute		–90.1 (–120.9, –9.3)	<0.0001
		%		–55.8 (–75.4, –36.2)	<0.0001
	Nuclear	Absolute		6.9 (–10.7, 24.6)	0.42
		%		8.9 (–258.2, 276.2)	0.94
Secondary					
pAKT (H-score)					
	Total	Absolute		81.3 (27.1, 135.5)	0.005

		%	116.9 (29.3, 204.6)	0.011
	Cytoplasmic	Absolute	24.8 (–7.51, 57.1)	0.127
		%	76 (–3.3, 155.2)	0.0595
	Nuclear	Absolute	53 (13.2, 92.8)	0.011
		%	516.5 (–29.7, 1062.6)	0.063
pS6 (H-score)				
	Cytoplasmic	Absolute	–42.3 (69.7, –14.8)	0.004
		%	–30 (–48.8, –11.1)	0.003
FOXO3a (H-score)				
	Total	Absolute	29.6 (–19.9, 79.2)	0.229
		%	19.4 (–21.4, 60.2)	0.338
	Cytoplasmic	Absolute	–46.2 (–77.0, –15.4)	<0.0001
		%	–42.4 (–97.2, 12.5)	0.124
	Nuclear	Absolute	79.3 (35.8, 122.8)	0.0009
		%	843.5 (155.5, 1531.5)	0.018

[a]Mixed model: difference between placebo (n=11) and capivasertib (n=17) corrected for baseline values; [b]Adjusted for baseline value

B) Mean change from baseline for all doses of capivasertib (stages 1 and 2)

		Mean change from baseline (95% CI)		
		Capivasertib 480 mg bid (n=17)	**Capivasertib 360 mg bid (n=5)**	**Capivasertib 240 mg bid (n=7)**
Primary				
Ki67 (% cells positive)				
Nuclear	Absolute	–12.4 (–18.9, –5.92)	0.92 (–7.54, 9.38)	0.2 (–9.0, 9.3)
	%	–38.3 (–57.6, –19.1)	–0.33 (–30.3, 29.6)	21.6 (–15.7, 58.8)
pGSK3β (H-score)				
Total	Absolute	–66.9 (–96.3, –37.6)	–18.6 (–47. 8, 10.6)	–39.2 (–98.9, 20.6)
	%	–41.6 (–57.5, –25.8)	–27.1(–74.2, 20.0)	–9.30 (–94.9, 76.4)
Cytoplasmic	Absolute	–62.4 (–89.4, –35.3)	–18.6 (–47.8, 10.6)	–21.57 (–86.1, 43.0)
	%	–40.7 (–56.5, –25.0)	–27.1 (–74.2, 19.9)	192.0 (–305.4, 689.5)
Nuclear	Absolute	–4.6 (–10.9, –1.73)	0	0.14 (–0.21, 0.49)
	%	–37.1 (–61.3, –12.8)	0	0
pPRAS40 (H-score)				
Total	Absolute	–71.3 (–93.9, –48.7)	–69.6 (–11.2, –28.0)	–43.8 (–94.0, 6.3)
	%	–43.7 (–56.7, –30.7)	– 44.9 (–81.7, –8.12)	–28.3(–65.6, 8.9)
Cytoplasmic	Absolute	–76.5 (–103.3, –49.6)	–71.0 (–114.6, 27.5)	–35.3 (–94.1, 23.5)
	%	–51.2 (–65.4, –37.0)	–46.9 (–86.6, –7.29)	350.7 (–587.6, 1288.9)
Nuclear	Absolute	5.2 (–8.2, 18.5)	1.4 (–4.0, 6.78)	11.3 (–6.02, 28.6)
	%	105.6 (–42.2, 253.4)	200 (–546.4, 946.4)	340 (–405.2, 185.2)
Secondary				
pAKT (H-score)				
Total	Absolute	70.4 (23.2, 117.5)	79 (–5.7, 163.7)	106 (39.8, 172.2)
	%	107.0 (25.2, 188.9)	71.3 (–19.9, 162.4)	133.5 (21.8, 245.2)
Cytoplasmic	Absolute	24.82 (–0.7, 50.35)	26 (–17.99, 69.99)	54.3 (18.5, 90.1)
	%	69.7 (0.43, 139)	43.6 (–43.7, 130.9)	119 (–10.2, 227.8)

Nuclear	Absolute	45.5 (12.0, 78.8)	28.4 (1.86, 54.9)	55.3 (6.8, 103.8)
	%	508.2 (44.9, 971.4)	918.8 (–747.4, 2584.9)	1173.9 (–81.7, 2429.5)
pS6 (H-score)				
Cytoplasmic	Absolute	–63.1 (–81.5, –44.6)	–51.8 (–92.0, –11.6)	–66.4 (–107.3, –25.5)
	%	–38.8 (–48.8, 28.9)	–40.5 (–83.3, 2.3)	–21.2 (–92.3, 49.8)
FOXO3a (H-score)				
Total	Absolute	4.18 (–30.0, 38.3)	6.0 (–24.2, 36.2)	–21.4 (–74.1, 31.2)
	%	12.1 (–13.5, 37.6)	–12.8 (–73.6, 47.9)	–23.1 (–118.9, 72.6)
Cytoplasmic	Absolute	–65.6 (–96.9, –34.3)	34.4 (–20.5, 89.3)	35.0 (–7.5, 77.5)
	%	–40.2 (–76.4, –3.91)	144.9 (–218.0, 507.8)	145.3 (–54.7, 345.2)
Nuclear	Absolute	69.8 (27.9, 111.6)	34.4 (–20.5, 89.3)	35 (–7.47, 77.5)
	%	924.8 (307.9, 1541.7)	144.9 (–218.0, 507.8)	145.3 (–54.7, 345.2)

CI, confidence interval

Table 3. Treatment-emergent adverse events (all grades) observed at a frequency of ≥2 events in any treatment arm within 7 days of first dosing (safety analysis set)

	Stage 1		Stage 2		All
	Placebo (n=17)	Capivasertib 480 mg bid (n=19)	Capivasertib 360 mg bid (n=5)	Capivasertib 240 mg bid (n=7)	capivasertib doses (n=31)
Number of patients experiencing a TEAE,[a] n (%)	10 (59)	15 (79)	5 (100)	7 (100)	27 (87)
Total number of TEAEs observed,[a] n	23	58	11	9	78
Number of TEAEs observed by preferred term, n (%)[b]					
Diarrhea	3 (13.0)	11 (19.0)	1 (9.0)	1 (11.1)	13 (16.7)
Nausea	3 (13.0)	6 (10.3)	1 (9.0)	1 (11.1)	8 (10.3)
Headache	0	4 (6.9)	0	0	4 (5.1)
Dizziness	0	3 (5.2)	1 (9.0)	0	4 (5.1)
Proteinuria	1 (4.3)	3 (5.2)	0	0	3 (3.8)
Fatigue	1 (4.3)	2 (3.4)	0	1 (11.1)	3 (3.8)
Vomiting	1 (4.3)	2 (3.4)	0	0	2 (2.6)
Pain in extremity	0	2 (3.4)	0	0	2 (2.6)
Seroma	0	0	2 (18.2)	0	2 (2.6)
Constipation	2 (8.7)	1 (1.7)	0	0	1 (1.3)
Stomatitis	2 (8.7)	0	0	0	0

[a]Treatment emergent is defined as occurring within 7 days of first dosing or before scheduled chemotherapy; [b]Percentage per dose = (number of TEAEs by preferred term / total number of TEAEs observed) x 100

Figures

Figure 1. A) Heat map for stages 1 and 2 showing changes in primary and secondary biomarkers by individual patient. B) Examples of immunohistochemistry staining images

A) Green denotes a change in the direction expected with AKT pathway inhibition using capivasertib. Notably, capivasertib induces increases in pAKT because of its ATP competitive mechanism of action. Patients are ranked in this figure by percentage change in Ki67 (nuclear percentage positivity); bars and numbers represent percentage change from baseline (except for pAKT, where bars are capped at –100% and +100% change for presentation purposes). B) Original magnification of staining images was 20 x

Figure 2. Scatter plot matrices comparing the percentage changes in primary and secondary biomarkers for capivasertib 480 mg bid and placebo

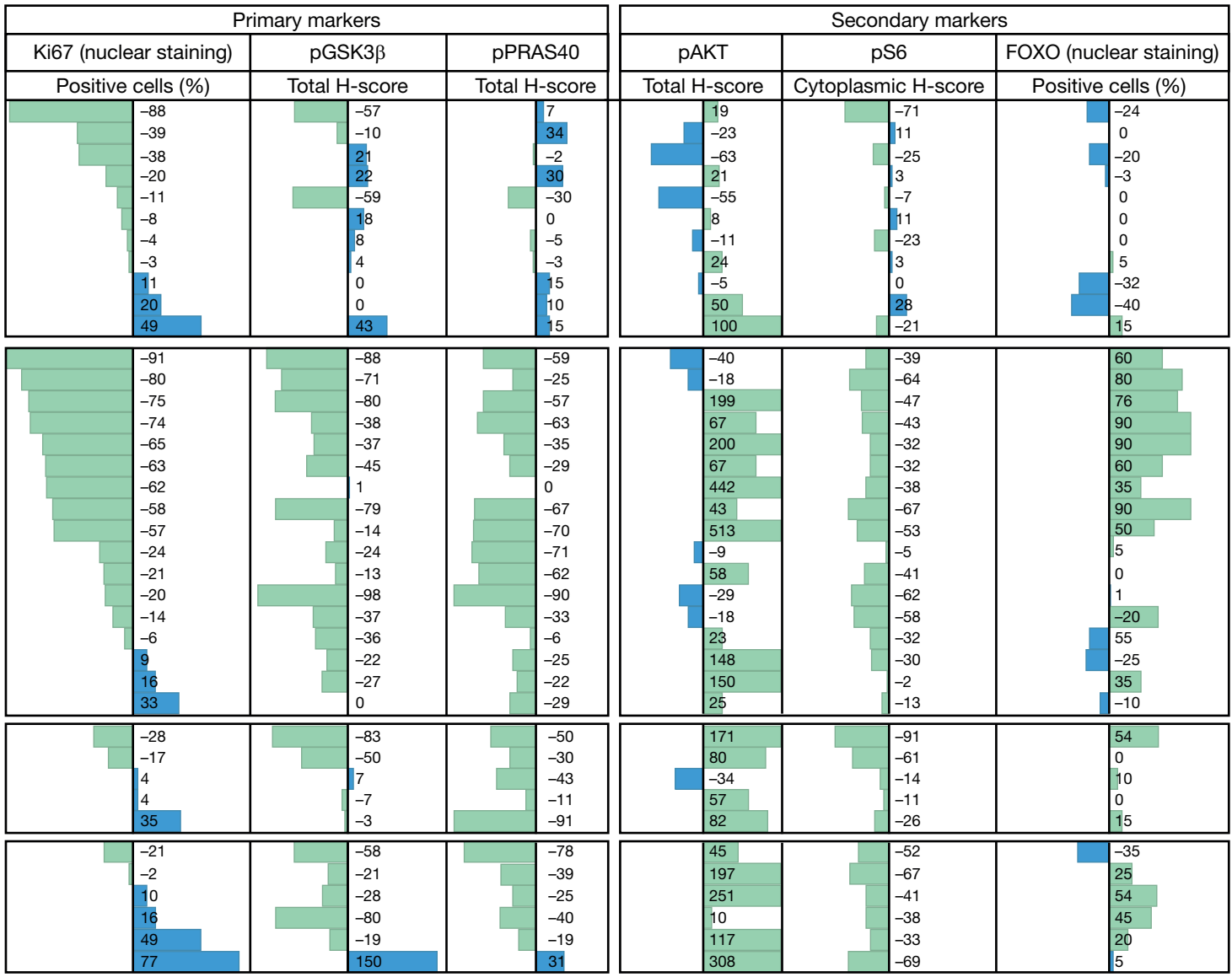
Nuclear score: Ki67 and FOXO3a; total score: pGSK3β, pPRAS40 and pAKT; cytoplasmic score: pS6. Relationships between biomarkers are quantified by Spearman's rank correlation coefficient (R) with *P* value. The red and blue solid lines represent the linear regression lines for capivasertib and placebo, respectively, and the red and blue zones represent 95% CI

Figure 3. Relationship between A) capivasertib dose and C_{max} on day 1 and B) capivasertib dose and capivasertib plasma concentration on day 5 at time of biopsy

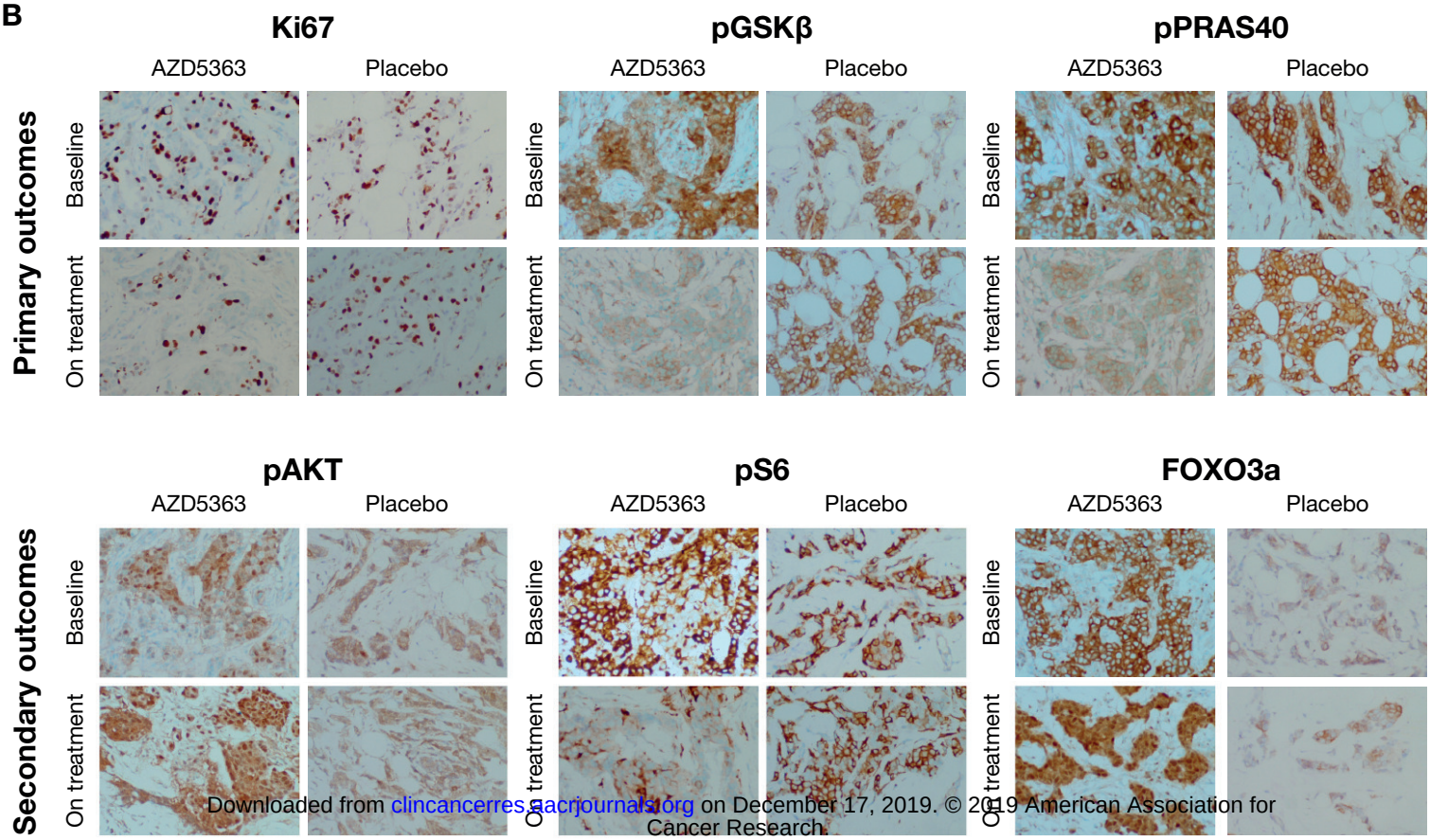
Figure 4. Observed and model-predicted dose– and exposure–response relationships for A) pGSK3β, B) pPRAS40 and C) Ki67 as percentage change from baseline

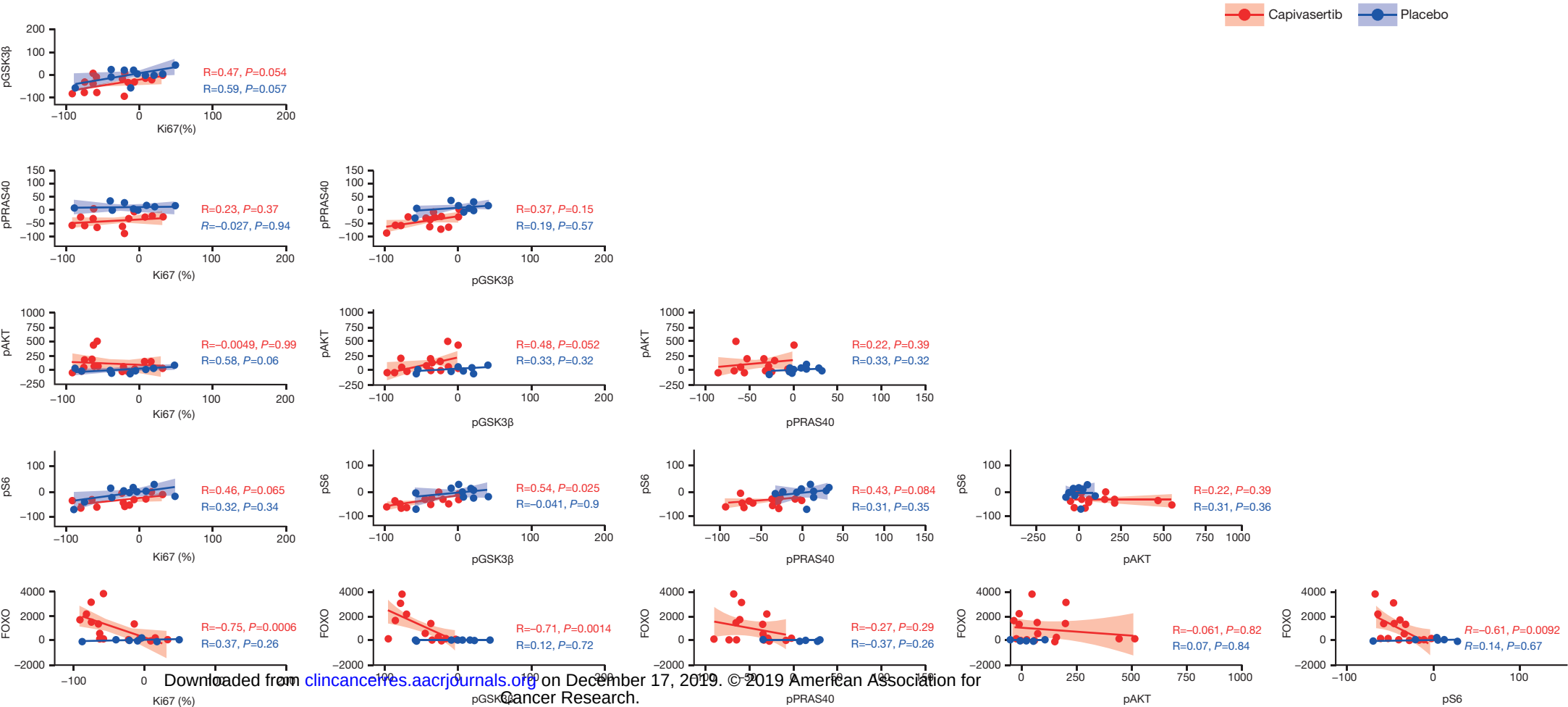
Day 5 concentrations are at the time of biopsy

A



B





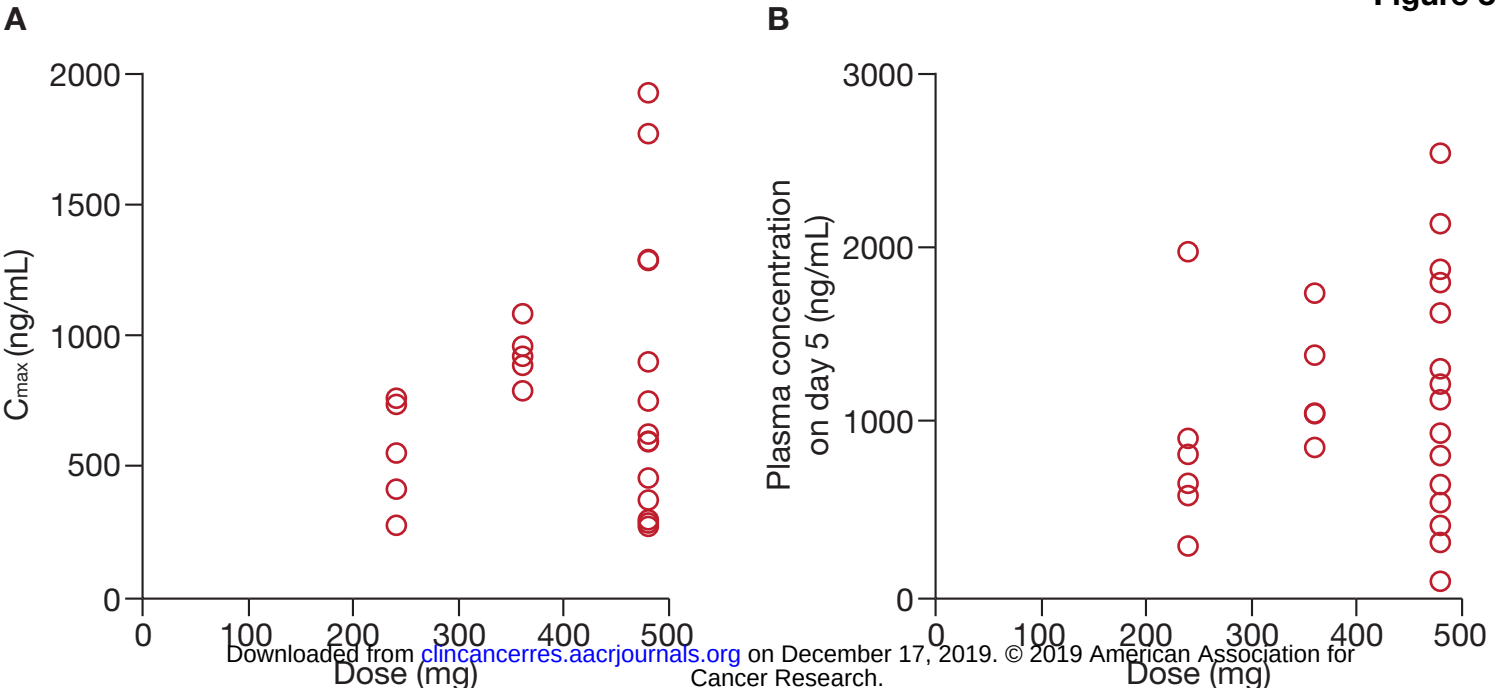
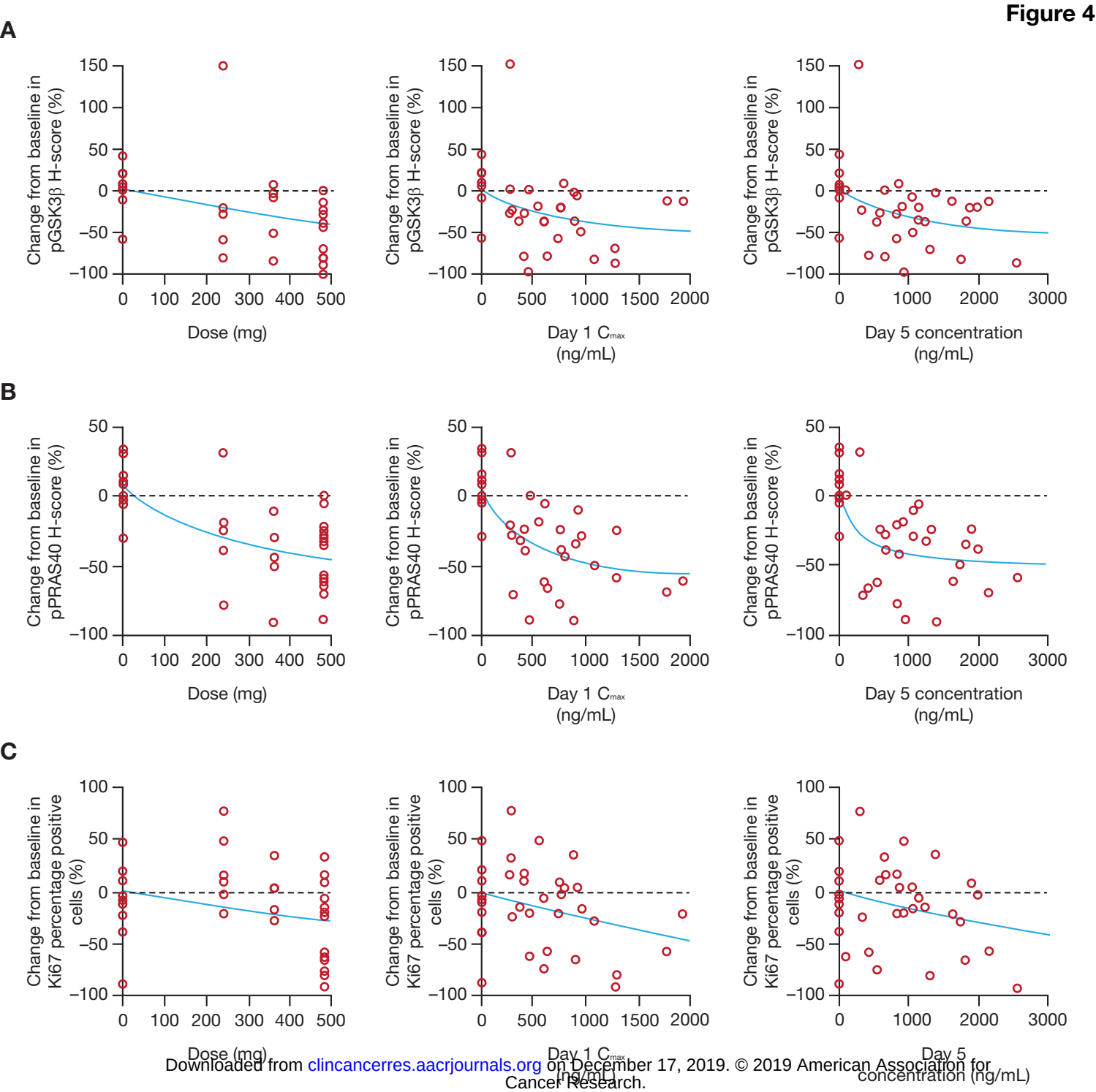


Figure 4



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Proliferation and AKT activity biomarker analyses after Capivasertib (AZD5363) treatment of patients with ER+ invasive breast cancer (STAKT)

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